Research Article

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Multi-drug Resistant Bacteria Isolated from Pus and Phenotypic Detection of Metallo β-lactamase Activity of *Pseudomonas Aeruginosa*

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ABSTRACT

Introduction: Pus is an exudate, present at the site of inflammation occurring during bacterial, viral or fungal infection and is formed by pyogenic bacteria.

Objective: To study the multi-drug resistant bacteria including phenotypic detection of MβL activity of Pseudomonas aeruginosa in pus isolates.

Methods: This was hospital based cross-sectional study in which 179 samples of pus was collected from any site of the body either by using swab or aspirated by using syringe and cultured in respective hospitals. Thus, obtained isolates were preserved and were transported to the laboratory for further processing.

Results: Out of 162 pus isolated, 67(42.0%) were Methecillin resistant *Staphylococcus aureus* (MRSA), 14(8%) were Methicillin sensitive *Staphylococcus aureus* (MSSA), 21(13.0%) were *Klebsiella spp*, 19(11.7%) were *Escherichia coli*, 14 (8.6%) were *Pseudomonas aeruginosa*, 10(6.2%) were *Acinetobacter spp*, 5(3.1%) were *Streptococcus spp*, 4(2.5%) were *Enterococci* ,3(1.9%) were *Enterobacter spp*, 2(1.2%) were Coagulase negative *S. aureus*, 1(0.6%) were *Proteus vulgaris*, and1(0.6%) were *Citrobacter spp*. Antibiogram of Gram positive cocci revealed that they showed susceptibility towards vancomycin, clindamycin, gentamycin, amikacin. Similarly, Gram negative bacilli showed good response towards gentamycin, amikacin, nitrofurantoin, colistin. Out of 162 isolates, 102(62.96%) were Multi-Drug Resistant (MDR) and 60 (37.04%) were Non-MDR. Out of 14 (8.6%) *Pseudomonas aeruginosa* isolates, 8(57.1%) were Imepenem resistant and among imepenem resistant, only 3(37.5%) of them showed MβL production activity.

Conclusion: This study showed that Gram negative bacteria were highly resistant to antibiotics. *Staphylococcus aureus* was found to be more predominant bacteria in forming pus. Antibiotics used in the infections related to pus are being more resistant.

Keywords: Antibiotics; Metallo β-lactamase; Multidrug resistant; Pus.

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INTRODUCTION

Pus is an exudate, present at the site of inflammation occurring during bacterial, viral or fungal infection and is formed by pyogenic bacteria, which can produce the accumulation of dead leukocytes and infectious agent.[1,2]

The most common aerobic pus producing Gram positive bacteria are *Staphylococcus aureus* and *Streptococcus spp*. and Gram-negative bacteria are

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Klebsiella spp., Pseudomonas spp., Escherichia coli, Enterobacter, Providencia, Acinetobacter, in which *Staphylococcus. aureus* is the most frequent bacteria.[3]

Antibiotics sensitivity test (AST) is the susceptibility of bacteria to antibiotics which is usually carried out to determine suitable antibiotics in treating a bacterial infection *in-vivo*.[4]

Multidrug resistance in bacteria may occur by one of two mechanisms. First, these bacteria may create multiple genes, each coding for resistance to a single drug, within a single cell on resistance (R) plasmids. Second, it may also occur by the increased gene expression coding different multidrug efflux pumps, expelling variety of drugs.[5,6]

The present study was designed to evaluate the profile of aerobic pyogenic bacteria along with their susceptibility to antimicrobial agents including phenotypic detection of Metallo Beta-Lactamase (MβL) activity of *Pseudomonas aeruginosa* in pus isolates.

Methods

It was a hospital based cross-sectional descriptive study from 16th October 2016 AD to 15th December 2016 AD, Nepal. Pus sample were collected from various hospitals of Pokhara valley. All the age groups were included in the study. Samples were collected from the limited hospitals of the Pokhara Valley. The sample size was calculated based on the approximate number of patients visiting 4 different hospitals where they were recommended for pus culture. Keeping 95% confidence level and 5% confidence interval, we estimated that there were at least 500 patients. So, by using the above parameters, we determined our sample size to be 217. Total 179 samples of pus was collected from any site of the body either by using swab or aspirated by using syringe and cultured in respective hospitals. Thus, obtained isolates were preserved in the vials containing glycerol (16%) and Brain Heart Infusion Broth (84%) and were transported to the Laboratory of School of Health and Allied Sciences, Pokhara University for further processing. Isolates were next asceptically inoculated onto Nutrient Agar, Blood Agar and MacConkey Agar plate with standard inoculation loop (4 mm) using Quadrant Streaking Technique and incubated for 24 hours at 37°C. Colony morphology was studied for shape, size, elevation, margin, surface, edge, colour, transparency and consistency. MacConkey plate was observed for the utilization pattern of lactose as lactose fermenter (LF) and Non-Lactose fermenter (NLF) and type of hemolysis was observed in Blood Agar plate (BA) namely alpha (greenish color around the colony), beta (clear zone around the colony) and gamma hemolysis (no detectable hemolysis). Out of 179 samples, there was no growth of 25 samples in culture media in which 154 samples showed growth. Among 154 samples, 162 isolates were obtained and 9 samples showed polymicrobial growth. Gram staining was performed for the differentiation of gram positive and gram negative bacteria, gram positive cocci (GPC) were further subjected to one step enzymatic test like catalase and coagulase (free and bound coagulase). Gram negative bacilli (GNB) were only enrolled for biochemical testing and identified by the different biochemical reaction patterns exhibited in different media like: Oxidative and Fermentative (OF) media, Sulphur Indole Motility (SIM), Urease media, Citrate media, and Triple sugar iron (TSI) media, Oxidative test was carried out for confirmation of aerobic gram negative bacteria.

For the Phenotypic detection of MβL activity, test organism was inoculated onto plates of Mueller-Hinton agar plate (opacity adjusted to 0.5 McFarland opacity standards).[7] A 0.5-m EDTA solution was prepared by dissolving 186.1 g of disodium EDTA 2H 2O in 1000 ml of distilled water and adjusting it to pH 8.0 by using 1% NaOH. The mixture was sterilized by autoclaving. Two 10-µg imipenem discs were placed on the plate; 5 µl of EDTA solution was added to one of the disc each. The inhibition zones of the imipenem and imipenem-EDTA discs were compared after 16-18 h of incubation at 35°C. An increase in the zone size of at least 7 mm around the imipenem-EDTA disc was recorded as an MβL-positive strain.[7,8] Data was entered and analysis was done by Microsoft excel, SPSS V20.0 software.

Results

As shown in Table 1, highest occurrence was seen in male (54.3%) as compared to female (45.7%).

Table 1: Association between sex and specific bacterias

Bacteria	Sex		Total	p value
	F	M		
Acinetobacter	7 (4.3%)	3 (1.9%)	10 (6.2%)	0.089
Citrobacter	1 (.6%)	0 (.0%)	1 (.6%)	0.500
E.coli	10 (6.2%)	9 (5.6%)	19 (11.7%)	0.500
Enterobacter	2 (1.2%)	1 (.6%)	3 (1.9%)	0.500
Enterococci	1 (.6%)	3 (1.9%)	4 (2.5%)	0.243
K.oxytoca	1 (.6%)	4 (2.5%)	5 (3.1%)	0.103
K.pneumoniae	6 (3.7%0	10 (6.2%)	16 (9.9%)	0.144
MRSA	29 (17.9%)	38 (23.5%)	67 (41.4%)	0.115
MSSA	9 (5.6%)	5 (3.1%)	14 (8.7%)	0.217
Micrococci	1 (.6%)	0 (0%)	1 (.6%)	0.500
CONS	0 (0%)	2 (1.2%)	2 (1.2%)	0.500
P. vulgaris	1 (.6%)	0 (.0%)	1 (.6%)	0.500
Pseudomonas	4 (2.5%)	10 (6.2%)	14 (8.6%)	0.028
Streptococcus	2 (1.2%)	3 (1.8%)	5 (3.1%)	0.500
Total	74 (45.7%)	88 (54.3%)	162 (100.0%)	

As shown in Table 2 all bacteria were resistant to ceftazidine whereas 57 isolates were sensitive to amikacin followed by vancomycin (53 isolates). Likewise, in case of 14 isolates of MSSA, all the isolates were resistant to ceftazidime. In case of 5 isolates of Streptococcus spp., all the isolates were sensitive to amikacin, vancomycin and gentamycin. The results were interpreted according to CLSI criteria.

Table 2: Antibiotics Sensitivity Patterns of Gram-Positive Bacteria

Bacteria	MRSA-67			MSSA-14			Streptococcus spp-5		
Antibiotics	S	I	R	S	I	R	S	I	R
Amikacin	57	6	4	4	0	10	5	0	0
Vancomycin	53	1	13	14	0	0	5	0	0
Cefoxitin	0	5	62	14	0	0	-	-	-
Oxacillin	0	0	67	14	0	0	-	-	-
Erythromycin	20	1	47	8	2	4	4	1	0
Clindamycin	32	9	26	11	2	1	4	0	1
Gentamycin	47	10	10	13	0	1	5	0	0
Ceftazidime	0	0	67	0	0	14	-	-	-
Ciprofloxacin	29	9	29	6	3	5	2	2	1

With MRSA AND MSSA p value <0.05
Hint: S= Sensitive, I= Intermediate, R= Resistant

As shown in Table 3 Gram negative bacteria were most susceptible to amikacin followed by gentamicin.

Table 3: Antibiotics Sensitivity Patterns of Gram-Negative Bacteria

Bacteria	Ecoli- 19			Psedomonas-14			Acinetobacter-10			Klebseilla-21		
Antibiotics	S	I	R	S	I	R	S	I	R	S	I	R
Amikacin	17	0	2	13	0	1	5	0	5	18	1	2
Gentamicin	18	1	0	13	0	1	9	1	0	13	3	5
Ceftriaxone	4	0	15	-	-	-	-	-	-	0	3	18
Nalidixic	4	1	14		-	-	-	-	-	7	2	12
Piperacillin	17	0	2	-	-	-	-	-	-	9	5	7
Ceftazidime	0	0	19	0	0	8	4	0	0	0	0	21
Vancomycin	-	-	-	-	-	-	4	0	6	-	-	-
Ciprofloxacin	-	-	-	1	13	0	1	2	7	5	5	11
Colistin	-	-	-	14	0	0	8	1	1	-	-	-
Tertacycline	13	0	6	-	-	-	6	0	4	12	1	8
Imepenem	-	-	-	6	0	8	4	1	5	-	-	-
Levofloxacin	-		-	2	1	11	-	-	-	-	-	-

Hint: S = sensitive, I= intermediate, R= resistant

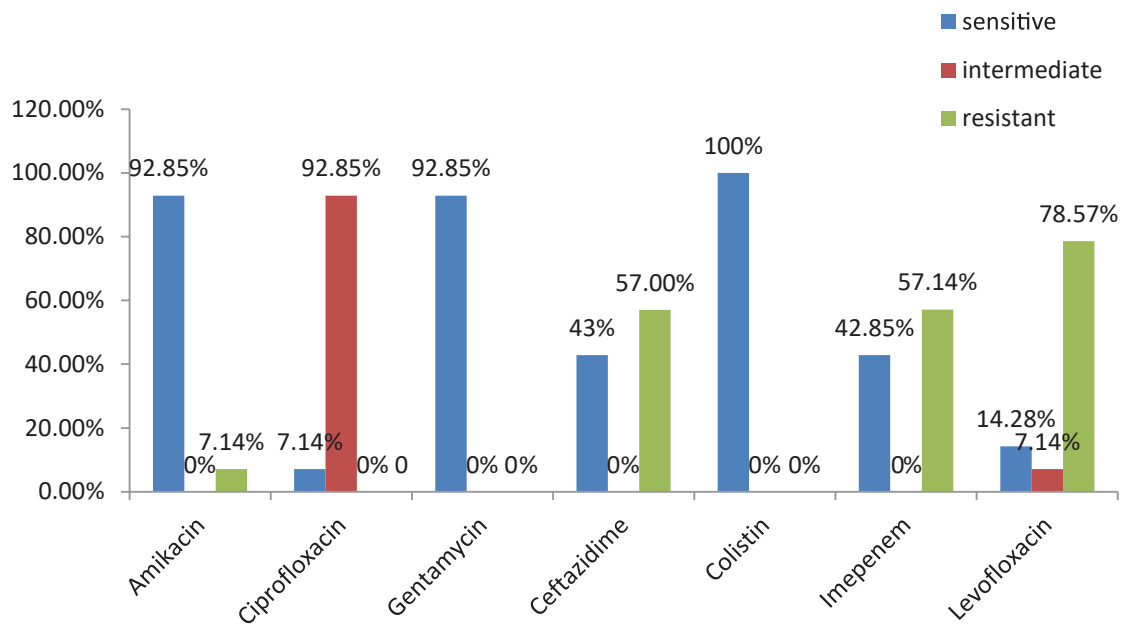

Figure 1: AST pattern of Pseudomonas

As shown in figure 1 all Pseudomonas aeruginosa isolates were resistant to levofloxacin (78.57%), imepenem (57.14%) and ceftazidime (57.0%). Pseudomonas isolates are 100% sensitive to Colistin followed by Amikacin (92.85%), gentamycin (92.85%). 92.85% of *Pseudomonas aeruginosa* are neither sensitive nor resistant (intermediate) to ciprofloxacin.

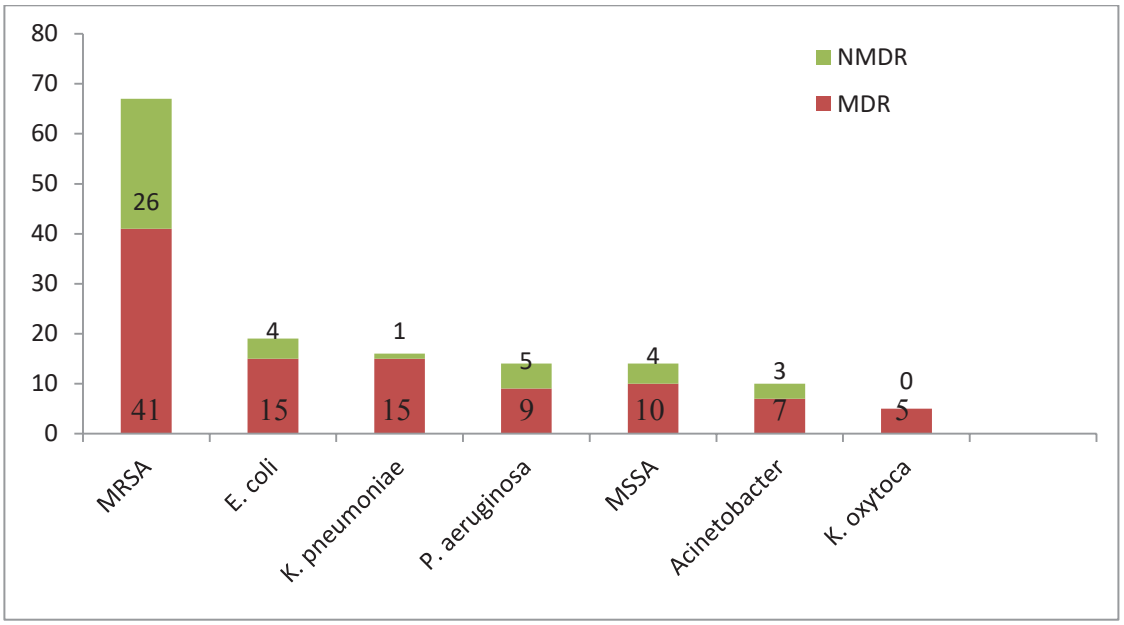

Figure 2: MDR and NMDR bacteria

Figure 2 shows that most of the bacteria isolated from pus were MDR and few were NMDR.

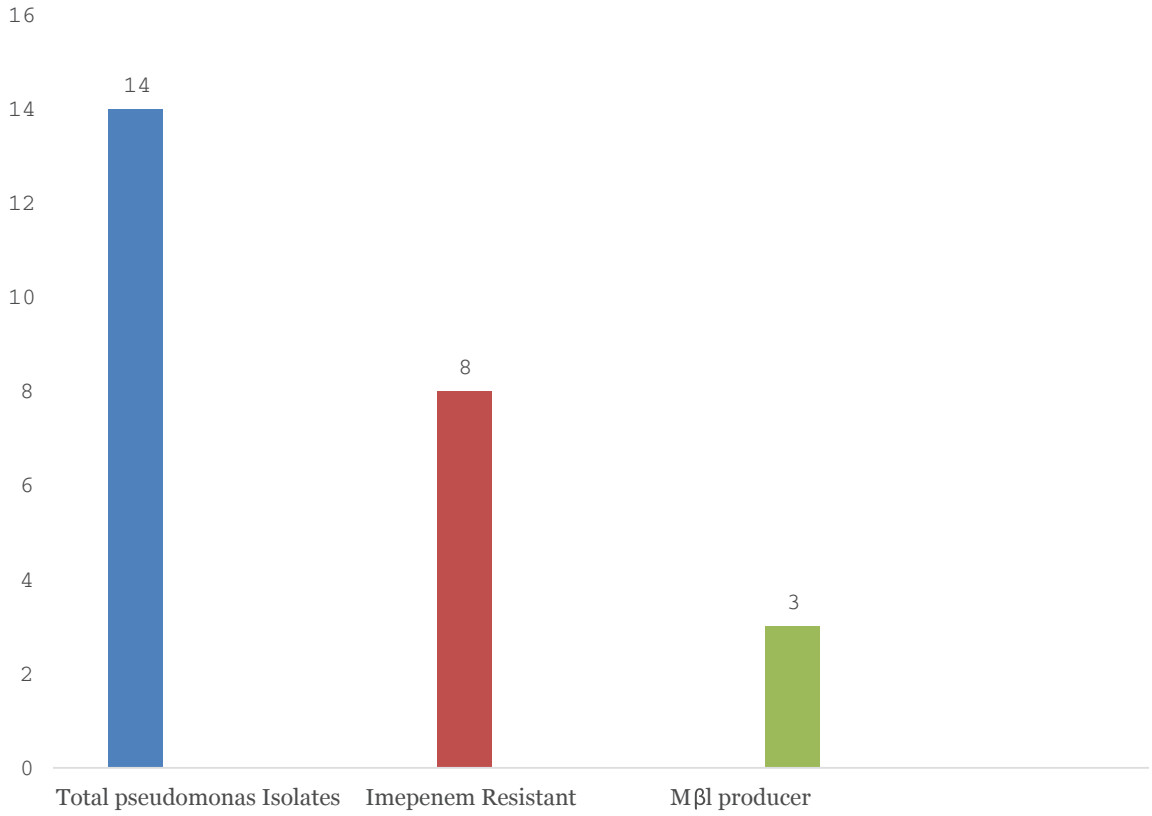

Figure 3: Shows number of imipenem resistant and metallo-beta lactamase +ve P. aeruginosa isolates

Figure 3 shows that out of 14 pseudomonas isolates, 8 were imepenem resistant and among imepenem Resistant isolates, only 3 of them show MβL production activity.

Discussion

It was a hospital based cross-sectional descriptive study. Pus isolates were collected from various hospitals of Pokhara valley, Nepal.

In our study highest occurrence were observed in male (49.7%) as compared to female (40.7%). It was correlated with Sharma V et. al.,[9] study which shows male preponderance 55 (55%) than Females 45 (45%). The majority of the isolates were in the age range 15-25 years (p= <0.05) followed by highest through 60 years which is similar to the study done by Muluye D et.al.[10] Out of 162 cultures smears 57.40% were gram positive and 42.59% were gram negative. Our study agrees with similar study done by Chaudhary P. et.al.[11]

Gram positive cocci such as *Staphylococcus aureus*, *Streptococcus spp*. and Gram-negative bacteria such as *Pseudomonas aeruginosa, Escherichia coli, Klebsiella spp*, *Acinetobacter spp* are the common causative agents of various pyogenic infections. Out of 162 pus isolates, 67(42.0%) were MRSA, 14(8%) were MSSA, 21(13.0%) were *Klebsiella spp.* , 19(11.7%) were E.coli, 14 (8.6%) were *Pseudomonas aeruginosa*, 10(6.2%) were *Acinetobacter spp*, 5(3.1%) were *Streptococcus spp*, 4(2.5%) were *Enterococci*, 3(1.9%) were *Enterobacter* spp, 2(1.2%) were CONS , 1(0.6%) were *Proteus vulgaris*, and 1(0.6%) were *Citrobacter* spp. Of total isolates, 102(62.96%) were MDR and 60 (37.04%) were Non-MDR. Similar Study was carried out by Grace BN et. al, which showed *Klebsiella pneumoniae* was predominant GNB followed by *Pseudomonas , Escherichia coli and Proteus.* [12]

Anti biogram of gram-positive cocci revealed that they showed susceptibility towards Vancomycin, clindamycin, gentamycin, but resistance towards penicillin and cefoxitin Whereas gram negative bacilli showing good response towards gentamycin, amikacin, nitrofurantoin, colistin. All these studies were in agreement with the study by Suguneswari et al.[13] Most of the gram-negative bacilli were resistant to ceftazidime, norfloxacin, ciprofloxacin and ceftriaxone.

Staphylococcus aureus (50%) was the most common gram positive isolate. These findings are similar to the results obtained by Zafar A et al (2008) [14] where *S. aureus* was isolated as the predominant species (41.28%) in Lahore. Similar Study was carried out by Lakhey M et. al (2007) [15] where S. aureus was the predominant organism accounting 50% of total bacterial species. *Klebsiella* spp. (12.96%) was the most common Gram-negative isolate which is in accordance with the report of Khinde AO et.al.[16]

Almost all *Staphylococcus aureus* isolates were sensitive to Aminoglycosides and Vancomycin. Out of 81 *S. aureus*, 68(82.71%) isolates were MRSA and 13(17.28%) isolates were MSSA. Only 2 pus isolates were isolated as CONS. Our study showed in accordance to the study done by Sapkota et. al., where out of 133 Staphylococcus aureus, 94 (70.64%) were MRSA.[17]

In our study 21 *Klebsiella* spp. were isolated, of which 16(76.19%) were *K. pneumoniae* and remaining 5(23.8%) were *K.oxytoca*. Most of the *Klebsiella* spp. were resistant to Ceftazidime, Ciprofloxacin, Ceftriaxone and Nalidixic acid and sensitive to Amikacin, Gentamycin and Norfloxacin. The study done by Thapa P et.al shows that Amikacin is the most sensitive drug for *Klebsiella* spp.[18]

Moreover 1(0.6%) species of *Micrococcus*, and the least commonly isolated species from pus isolates was *Citrobacter* spp. 1(0.62%). Similar result was observed by Patil et. al., 2006 19, in Mumbay. *Proteus* species was found to be least prevalent which is correlated with the study done by Grace BN et.al.[12]

In our study, *P.aeruginosa* was isolated from 4(2.5%) females and 10(6.2%) males which is statistically significant (p=0.028). *P. aeruginosa* were sensitive to amikacin, gentamycin and colistin which corresponds with the results obtained by Thapa P et.al.[18] All Pseudomonas isolates were resistant to Ceftazidime. Out of 14 *P. aeruginosa* isolates, 8 were resistant to Imepenem and remaining 7 were sensitive. Imepenem resistant *P. aeruginosa* were suspected for MβL production and 3(37.5%) of them were confirmed as MβL producers by Combined Disk Method which is in agreement with the study done by Ansari et.al., where out of 178 *P. aeruginosa* isolates, MβLs mediated resistance was observed in 55 (30.9%) isolates.[20]

In our study, due to limited time period, the sample

size was less and the study was done only on pus sample.

Conclusions

The incidence of multidrug resistant and extensively drug resistant organisms is rapidly increasing, especially among Gram negative bacteria. This may necessitate a revision of antibiotic policy for infected patients. Out of 179 samples, 25 showed no growth and 154 sample showed growth, of which 162 isolates were obtained including 9 polybacterial, of which prevalence of male was higher than female. Isolates obtained were *S.aureus, Klebsiella, E.coli, Pseudomonas aeruginosa, Acinetobacter spp, Citrobacter spp, Streptococcus spp, Micrococci*, CONS and *Proteus spp*. *S aureus* is the most common bacteria obtained in pus isolates, followed by *Klebsiella spp*. Incidence of MRSA is fairly high. Gram positive were more resistant to cefoxitin, oxacillin while being most sensitive to vancomycin, gentamycin and clindamycin. Most of gram negative bacilli are resistant to ceftazidime, ceftriaxone, cipirofloxacin, while most being sensitive to gentamycin, amikacin, colistin, piperacillin. Some of the imepenem resistant P.aeruginosa shows MβL activity.

On the basis of our study the following recommendation are suggested,

- MRSA is the predominant cause of pyogenic infection
- Antibiotics that are commonly used for the management of pyogenic infection are being less effective.
- For instance cefoxitin, and penicillin are being highly resistant to pus isolates.
- Universal and rational recommendations might not reflect the local scenario so survelliance programs should be strengthened for the establishment of local antibiogram and should be constantly renewed over time.
- MβL detection showed the presence of MβL production in imepenem resistant P.aeruginosa that might harbor resistant genes.

Conflict of interest : None

References

1. Koneman EW, Allen SD, Janda WM, Schreckenberger PC, Winn WC. Diagnostic microbiology. The nonfermentative gram-negative bacilli. Philedelphia: Lippincott-Raven Publishers. 1997:253-320.
2. Ananthanarayan R. Ananthanarayan and Paniker's textbook of microbiology. Paniker CJ, editor. Orient Blackswan; 2005.
3. Kumar AR. Antimicrobial sensitivity pattern of Klebsiella pneumonia isolated from pus from tertiary care hospital and issues related to the rational selection of antimicrobials. Journal of chemical and pharmaceutical research. 2013;5(11):326-31.
4. Haque M, Singh AK, Maurya SK, Seth A. Formulation development, physico-chemical characterization and evaluation of anti-microbial activity of herbal tooth gel. J Chem Pharm Res. 2014;6(3):1279-85.
5. AlonCudkowicz N, Schuldiner S. Deletion of the major Escherichia coli multidrug transporter AcrB reveals transporter plasticity and redundancy in bacterial cells. PloS one. 2019 Jun 28;14(6):e0218828. https://doi.org/10.1371/journal.pone.0218828
6. Chakraborty P. Textbook of medical parasitology. New Central Book Agency; 2009.
7. Patil HV, Mohite ST, Patil VC. Metallo-beta-lactamase-producing multidrug-pesistant acinetobacter isolates in patients with ventilator-associated pneumonia. Journal of Natural Science, Biology and Medicine. 2021 Jan 1;12(1):64.
8. Todar, Kenneth G. Todar's Online Textbook of Bacteriology. Madison, WI: Kenneth Todar, University

of Wisconsin-Madison Dept. of Bacteriology, 2006. Internet resource.
9. Sharma V, Parihar G, Sharma V, Sharma H. A Study of Various Isolates from Pus Sample with Their Antibiogram from Jln Hospital, Ajmer. IOSR Journal of Dental and Medical Sciences (IOSR-JDMS). 2015;1(14):64-8.
10. Muluye D, Wondimeneh Y, Ferede G, Nega T, Adane K, Biadgo B, et al. Bacterial isolates and their antibiotic susceptibility patterns among patients with pus and/or wound discharge at Gondar university hospital. BMC research notes. 2014 Sep 9;7(1):619.
11. Chaudhary P, Shakya C, Pokhrel SR, Karki S, Shrestha B, Timalsina B, et al. Prospective study on bacterial isolates with their antibiotic susceptibility pattern form pus (wound) sample in Kathmandu Model hospital. International Journal of Medicine & Biomedical Sciences. 2015;1(1):15-22.
12. Grace BN, Kiran KR, Rao BV. Study of Aerobic Bacterial Isolates and Their Antibiogram from Pus Sample in Government General Hospital, Guntur.
13. Rao DR, Basu R, Biswas DR. Aerobic Bacterial Profile and Antimicrobial Susceptibility Pattern of Pus Isolates in a South Indian Tertiary Care Hospital. Surgery. 2014;36:35-29.
14. Zafar A, Anwar N, Ejaz H. Bacteriology of infected wounds–A study conducted at children's hospital Lahore. Biomedica. 2008 Jan;24:71-4.
15. Lakhey M, Bhatt CP. The distribution of pathogens causing wound infection and their antibiotic susceptibility pattern. Journal of Nepal Health Research Council. 2008 Dec 30.
16. Kehinde AO, Ademola SA, Okesola AO, Oluwatosin OM, Bakare RA . Pattern of bacterial pathogens in burn wound infections in Ibadan, Nigeria. Annals of Burns and fire disasters. 2004;17(1):12-5.
17. Sapkota J, Sharma M, Jha B, Bhatt CP. Prevalence of staphylococcus aureus isolated from clinical samples in a tertiary care hospital: A descriptive cross-sectional study. JNMA: Journal of the Nepal Medical Association. 2019 Nov;57(220):398. DOI: https://doi.org/10.31729/jnma.4673
18. Thapa P, Bhandari D, Shrestha D, Parajuli H, Chaudhary P, Amatya J, et al. Hospital based surveillance of metallo-beta-lactamase producing gram negative bacteria in Nepal by imipenem-EDTA disk method. BMC research notes. 2017 Dec;10(1):1-6.
19. Patil R, Baveja S, Nataraj G, Khopkar U. Prevalence of methicillin-resistant Staphylococcus aureus (MRSA) in community-acquired primary pyoderma. Indian Journal of Dermatology, Venereology, and Leprology. 2006 Mar 1;72(2):126.
20. Ansari S, Dhital R, Shrestha S, Thapa S, Puri R, Chaudhary N, et al. Growing Menace of Antibacterial Resistance in Clinical Isolates of Pseudomonas aeruginosa in Nepal: An Insight of Beta-Lactamase Production. BioMed Research International. 2016 Aug 23; 2016. https://doi.org/10.1155/2016/643720